PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
G888-PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/JP 99/04238	05/08/1999	06/08/1998
Applicant		
TEIJIN LIMITED et. al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 99/04238

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 25-36 are directed to a method of treaetment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 99/04238

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/395 A61K38/17 A61K31/70 A61P13/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OCHIENG J ET AL: "Regulation of cellular adhesion to extracellular matrix proteins by galectin-3." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 MAY 29) 246 (3) 788-91. , XP002131316 the whole document ---	1-36
A	HENRICK K ET AL: "Evidence for subsites in the galectins involved in sugar binding at the nonreducing end of the central galactose of oligosaccharide ligands: sequence analysis, homology modeling and mutagenesis studies of hamster galectin-3." GLYCOBIOLOGY, (1998 JAN) 8 (1) 45-57. , XP000876954 the whole document ---	1-36
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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
23 February 2000	16/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	PROBSTMEIER R ET AL: "Galectin-3, a beta-galactoside-binding animal lectin, binds to neural recognition molecules." JOURNAL OF NEUROCHEMISTRY, (1995 JUN) 64 (6) 2465-72. , XP000876914 the whole document ---	1-36
A	LIU F T ET AL: "Modulation of functional properties of galectin -3 by monoclonal antibodies binding to the non-lectin domains." BIOCHEMISTRY, (1996 MAY 14) 35 (19) 6073-9. , XP002131317 cited in the application the whole document -----	1-36

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A61K 39/395, 38/17, 31/70, A61P 13/12

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(54) Title: PHARMACEUTICAL COMPOSITION HAVING INHIBITORY EFFECT ON OVERPRODUCTION AND ACCUMULATION OF EXTRACELLULAR MATRIX

(57) Abstract

A pharmaceutical composition having an inhibitory effect on the overproduction and the accumulation of extracellular matrix, said composition comprising as an active ingredient a compound that inhibits the biological activity of galectin–3, which pharmaceutical composition can serve as a therapeutic or preventive agent for glomerular nephritis, diabetic nephropathy or tissu fibrosis, as well as the use of said compound for the production of pharmaceuticals for the above–mentioned use, and a method for inhibition of the overproduction and accumulation of the extracellular matrix.

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DESCRIPTION

PHARMACEUTICAL COMPOSITION HAVING INHIBITORY EFFECT ON OVERPRODUCTION AND ACCUMULATION OF EXTRACELLULAR MATRIX

FIELD OF INVENTION

The present invention relates to a preventive or therapeutic pharmaceutical composition having an inhibitory effect on the overproduction and the accumulation of extracellular matrix, said composition comprising as an active ingredient a compound that inhibits the biological activity of galectin-3.

BACKGROUND ART

Galectin-3 is a protein that has a molecular weight of about 30 Kd belonging to the family of β-galactoside-binding protein and is a lectin that widely occurs on the cell surface, the cytoplasm and the nucleus of the tissue (see, for example, Barondes, S. H. et al., J. Biol. Chem. (1994) 296: 20807-20810, Hughes, R. C. Glycobiology (1994) 4: 5-12, Wang, L. et al., Biochem. Biophys. Res. Commun. (1995) 217: 292-303, and the like). It is known that galectin-3 binds to a suitable sugar chain portion of glycoprotein present on the cell surface or in the extracellular matrix (ECM) and thereby activates inflammatory cells such as neutrophils, basophils, or macrophages to promote the production of cytokines from these cells (see, for example, Sato, S. et al., J. Biol. Chem. (1994a) 269: 4424-4430, Liu, F. T. Immunol. Today (1993) 14: 486-490), or to suppress the apoptotic death of T cells by its overexpression (see Yang, R-Y, H. et al., Proc. Natl. Acad. Sci. U.S.A. (1996) 93: 6737-6742), and it is believed to be an important protein responsible for inflammatory and immunological reactions.

Furthermore, galectin-3 is also known to play an important role in the formation and repair of the tissue since it is highly expressed during the damage repair period in the rat lung-injured model induced by X-ray irradiation (see Kasper, M. et al., J. Pathol. (1996)

179: 309-316) and it is possibly playing an important role in the formation of kidney tissue in humans during the embryonic stage (see Winyard, P. J. D. et al., J. Am. Soc. Nephrol. (1997) 8: 1647-1657).

The overproduction and the accumulation of extracellular matrix (ECM) such as collagen is believed to be an important factor for the pathogenesis of the fibrosis of tissues such as liver, kidney, lung, heart, pancreas, artery, gastrointestinal tract, thyroid, salivary gland, and skin (see Okada, H. et al., Kidney Int. (1996) 49: Supple. 54: S-37-S-38, Coker, R, K. et al., Eur. Respir. J. (1998) 11(6) : 1218-1221 and the like). ECM is also involved in the maintenance of homeostasis of cellular functions together with the support of parenchymal cells at the physiological conditions. When minor injuries are inflicted to tissues, the repair of the injured tissues is completed by the treatment of the injured tissues by phagocytic cells in the process of inflammatory and repair reactions, the subsequent regeneration of parenchymal cells, and the reconstruction of the supportive substrate, ECM. However, when the injuries are severe or persist for a long time, the overproduction and the accumulation of ECM will cause severe damages in the functions of each tissue. In the liver, for example, lymphocytes and macrophages infiltrate to the periphery of the injured liver cells, and these cellular infiltrates and Kupfer cells or vascular endothelial cells and the like produce cytokines such as PDGF, TGF-β, etc., which then activate Ito cells, a kind of ECM-producing cells. The activated Ito cells proliferate and produce ECM in an excess amount in the Disse's space thereby to cause hepatic fibrosis or hepatic cirrhosis that are said to be a terminal status of the hepatic diseases. In the kidney glomeruli, for example, cytokines such as PDGF and TGF-β are produced from the

cells that have infiltrated into the periphery of the injured kidney cells or endothelial cells in the glomeruli etc. to activate the mesangium cells that are a kind of ECM-producing cells. The activated mesangium cells proliferate while themselves also producing cytokines such as PDGF and TGF-β together with an excessive amount of ECM, creating factors that cause various glomerular diseases, for example, chronic glomerular nephritis, including IgA nephropathy, diabetic nephropathy, glomerular sclerosis and the like. In the interstitial tissue of the kidney also, due to the activation of myofibroblasts, a kind of ECM-producing cells, and the epithelial cells of urinary tubules, these cells excessively produce ECM in the interstitial region of the urinary tubules and form fibrosis of the tubulo-interstitium thereby significantly reducing the renal function. Thus, the ECMs that were overproduced and accumulated in each tissue physically constrain the cellular functions of each cell and substitute for the functional unit of each tissue to cause severe functional disorders of each organ.

For the above-mentioned diseases, adrenal cortical steroids, immunosuppressive agents, anti-platelet agents, anti-coagulants, anti-fibrinolytic agents, ACE inhibitors, and the like are currently used, but no drugs exhibit satisfactory efficacy on the overproduction and the accumulation of ECM, and there is a strong need for agents that have a novel mechanism of action.

Although galectin-3 has been highly expressed at the injured site of the tissue in the rat lung-injured models induced by X-ray irradiation, a model of pulmonary fibrosis, it has not been elucidated whether it can regulate the overproduction and the accumulation of ECM such as collagen and the survival of the mesangium cells that are a kind of ECM-producing cells. Accordingly, it is not known whether the inhibition of the action of galectin-3 can inhibit the overproduction and the

accumulation of ECM and thereby it has a therapeutic and/or preventive usefulness for glomerular nephritis, diabetic nephropathy or tissue fibrosis.

DISCLOSURE OF INVENTION

It is an object of the present invention to provide a pharmaceutical composition having an inhibitory effect on the overproduction and the accumulation of ECM, said composition comprising as an active ingredient a compound that inhibits the biological activity of galectin-3. Furthermore, it is an object of the present invention to provide a therapeutic or preventive agent comprising said compound inhibiting the biological activity of galectin-3 and a pharmaceutically acceptable carrier. In particular, it is an object of the present invention to provide a therapeutic or preventive agent based on a novel mechanism of action of inhibiting the biological activity of galectin-3 for the diseases caused by the overproduction and the accumulation of ECM such as glomerular nephritis, diabetic nephropathy or tissue fibrosis for which no conventional drugs show satisfactory inhibitory effects.

Considering the state of art of the conventional technology, the present inventors have carried out intensive study and have found that galectin-3 is a molecule that can regulate the overproduction and the accumulation of ECM such as collagen and a molecule that can regulate the survival of the mesangium cells which is a kind of ECM-producing cells, and also have found that substances that inhibit the biological activity of galectin-3 can regulate the overproduction and the accumulation of ECM such as collagen, and have thereby completed the present invention.

Thus, the present invention provides a therapeutic or preventive pharmaceutical composition having an inhibitory effect on the overproduction and the accumulation of ECM, said composition comprising as an active ingredient a compound that inhibits the biological

activity of galectin-3, and a pharmaceutically acceptable carrier.

This indicates that compounds that inhibit the biological activity of galectin-3 can be a therapeutic or preventive agent of glomerular nephritis, diabetic nephropathy or tissue fibrosis of which cause is the overproduction and the accumulation of extracellular matrix.

BRIEF EXPLANATION OF THE DRAWINGS

Figure 1 shows a variation in the expression of galectin-3 in the anti-Thy-1.1 antibody-induced rat nephritis model.

A: the kidney of the rats who received no anti-Thy-1.1 antibody, B: day 8 after the administration of anti-Thy-1.1 antibody, C and D: day 14 after the administration of anti-Thy-1.1 antibody.

The asterisk in the figure shows a representative macula densa region, m a representative mesangium region, c a representative crescent body-forming region, the closed arrow tail a representative distal urinary tubule, the closed arrow a representative proximal urinary interstitial tubule, and the open arrow a representative infiltrated macrophage or fibroblast.

Figure 2 shows a variation in the expression of galectin-3 in the UUO rat model.

A: contralateral kidney, B: obstructed kidney

The asterisk in the figure shows a representative macula densa region, the closed arrow tail a representative distal urinary tubule, and the open arrow a representative infiltrated macrophage or fibroblast.

Figure 3 shows the activity of galectin-3 to inhibit the cellular death of the mesangium cells.

Figure 4 shows the activity of galectin-3 to promote the production of collagen type IV by the rat mesangium cells.

Figure 5 shows the suppression by galectin-3 binding-inhibiting glycoprotein of the activity of

galectin-3 to promote the production of collagen type IV production by the rat mesangium cells.

Figure 6 shows the suppression by galectin-3 binding-inhibiting sugar of the activity of galectin-3 to promote the production of collagen type IV production by the rat mesangium cells.

DETAILED DESCRIPTION

Compounds that inhibit the biological activity of galectin-3 for use in the present invention include, for example, the following:

(1) Anti-galectin 3 antibody: mouse anti-galectin 3 monoclonal antibody (for example, an antibody described in Lui, F. T., Et al., J. Biol. Chem. (1996) 35: 6073-6079);

(2) Inhibitors of galectin-3 binding: sugars to which galectin-3 can bind such as Galβ1-4Glc, Galβ1-4GlcNAc, Fucα1-2Galβ1-4Glc, Galα1-3Galβ1-4GlcNAc, Galβ1-3GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc, Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc, Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc, Galβ1-3GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc, Fucα1-2(GlcNAcα1-3)Galβ1-3GlcNAcβ1-3Galβ1-4Glc, NeuNAcα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc, NeuNAcα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galβ1-3(NeuNAcα2-6)GlcNAcβ1-3Galβ1-4Glc, Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galβ1-3GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-6(Galβ1-3GlcNAcβ1-3)Galβ1-4Glc, Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4Glc, Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAc, Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)Manα1-3)Manβ1-4GlcNAc, GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galα1-3Galβ1-4GlcNAcβ1-

3Galβ1-4Glc, GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc, Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galα1-3Galβ1-4GlcNAcβ1-6(Galα1-3Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galα1-3Galβ1-4GlcNAcβ1-6(Galα1-3Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-6(Galα1-3Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glc, Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAc, Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)Manα1-3)Manβ1-4GlcNAc, Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα1-6(Galβ1-4GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)Manα1-3)Manβ1-4GlcNAc, and blood type B-like sugar chains, or glycolipids comprising the above sugars, or glycoproteins having on the cell surface sugar chains to which galectin-3 can bind, or fragments thereof such as fetuin, asialofetuin, transferrin, asialotransferrin, α1-acid glycoprotein, asialo α1-acid glycoprotein, laminin, fibronectin, CD11b, Lamp-1, Lamp-2, Mac-3, CD98, neutrophil 115 kD protein, neutrophil 180 kD protein (NCA-160/CD66), high-affinity IgE receptor, Fc ε R1, and the like (see, for example, Feizi, T. et al., Biochemistry (1994) 33: 6342-6349, Sato, S., et al., J. Biol. Chem. (1992) 267: 6983-6990). Compounds or antibodies that inhibit the binding of galectin-3 and a sugar chain to which galectin-3 can bind;

(3) Compounds that inhibit the incorporation of galectin-3 into the cell: those which inhibit the biological activity of galectin-3 by acting on galectin-3 receptor or the cells that contain galectin-3 receptor, including, for example, antagonists of galectin-3 receptor, or anti-galectin 3 receptor antibody, AGE or AGE receptor or fragments thereof (see Vlassara, H. et

al., Molecular Medicine (1995) 1: 634-646, and the like);

(4) Compounds that inhibit the transfer of galectin-3 into the cell: inhibitors of galectin-3 transporter protein;

(5) Compounds that inhibit the biological activity of galectin-3 in the nucleus or in the cytoplasm: galectin-3 binding proteins that bind to galectin-3 in the nucleus or in the cytoplasm, or derivatives of nucleic acid or fragments thereof, or compounds that inhibit their binding; and

(6) Compounds that inhibit the expression or secretion of galectin-3: antisense of the galectin-3 gene, compounds that inhibit the function of the promoter region of the galectin-3 gene, compounds that inhibit the transfer of proteins in the cell such as brefeldin A.

Compounds that inhibit the biological activity of galectin-3 for use in the present invention can be formulated to make pharmaceutical compositions having an inhibitory effect on the overproduction and the accumulation of ECM by blending said compounds as active ingredients and pharmaceutically acceptable carriers. The pharmaceutical composition may be therapeutic or preventive agents comprising said compounds and pharmaceutically acceptable carriers.

Diseases caused by the overproduction and the accumulation of ECM include glomerular nephritis, diabetic nephropathy or tissue fibrosis and they also include glomerular nephritis, diabetic nephropathy or tissue fibrosis that are derived from the abnormal proliferation of the mesangium cells.

As used herein, pharmaceutically acceptable carriers can include those that are identical with the excipients mentioned below. The amounts blended of a compound that inhibits the biological activity of galectin-3 and a carrier, without any limitation, follow the dosage of the active ingredient mentioned below, and can be widely selected. The amount of a compound that inhibits the

biological activity of galectin-3 is usually 1 to 70 percent by weight and preferably 5 to 50 percent by weight in the total composition.

The composition thus obtained can be provided as an oral preparation such as a soft capsule, a hard capsule, a tablet, granules, powders, a suspension, a liquid, a syrup etc., an injection, a suppository, or an external preparation using a suitable excipient in a known method.

Such excipients include, for example, plant oils (for example, corn oil, cotton seed oil, coconut oil, almond oil, peanut oil, olive oil, and the like), oily esters such as glyceride oils of middle chain fatty acids, mineral oil, glycerin esters such as tricaprylin and triacetin, alcohols such as ethanol, physiological saline, propylene glycol, polyethylene glycol, vaseline, animal fats, cellulose derivatives (crystalline cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, methyl cellulose), polyvinylpyrrolidone, cyclodextrin, dextrin, lactose, mannitol, sorbitol, starch and the like.

The dosage of the active ingredient, though depends on the degree of the disease and the age of the patient etc., is about 0.01 mg to 1000 mg per day per capita, preferably 1 mg to 200 mg per day per capita. It is desired that the formulations satisfy such conditions.

EXAMPLES

The present invention will now be explained with reference to the following examples, but the present invention is not limited to these examples in any way.

Example 1. Variation in the expression of galectin-3 in the rat nephritis model induced by anti-Thy-1.1 antibody

Rabbit anti-Thy-1.1 antiserum was obtained by immunizing rabbits subcutaneously on the back with Thy-1.1 antigen purified from rat thymus cells. The rat nephritis model induced by anti-Thy-1.1 antibody was prepared by intravenously administering to the tails of

Sprague-Dawley rats 0.25 ml of the above rabbit anti-Thy-1.1 antiserum diluted 2-fold in phosphate buffered saline together with 0.25 ml of normal rabbit complement (Sigma) according to the method of Okuda et al. (Okuda S., et al., J. Clin. Invest. (1990) 86: 453-462). The rats were sacrificed on day 3, 7, and 14 after the administration of the antibody, and the kidney was extracted from each rat after perfusion with phosphate buffered saline. The extracted kidney was fixed in 4 % (w/v) phosphate buffered formalin, embedded in paraffin, and tissue sections for immunostaining were prepared. The immunostaining of the tissue sections was carried out by using affinity-purified rabbit anti-galectin-3 antibody as the primary antibody, peroxidase-labeled goat anti-rabbit antibody (Sigma) as the secondary antibody, and DAB (Sigma: DAB Tablet) as the chromogenic substrate.

The result of immunostaining of the tissue sections is shown in Figure 1. It was confirmed that in the rats who received no anti-Thy-1.1 antibody, a small quantity of galectin-3 was present in the distal urinary tubule and the macula densa region of the glomerulus, while in the rats who received anti-Thy-1.1 antibody, a large quantity of galectin-3 was observed in the distal and the proximal urinary tubule and the glomerulus on day 8 and 14 after the antibody administration. It was also confirmed that on day 14, infiltrated macrophage and fibroblasts were coexistent with galectin-3 in the pre-fibrotic region of the tubulo-interstitium. This finding confirmed that the expression of galectin-3 is increased at the time of pathogenesis in the rat nephritis models induced by anti-Thy-1.1 antibody, and since galectin-3 was coexistent with infiltrated macrophages or fibroblasts which are a kind of ECM-producing cells and are believed to induce the overproduction of ECM in the pre-fibrotic region, it was strongly suggested that galectin-3 is involved in the formation of fibrosis.

Example 2. Variation in galectin-3 expression in the unilateral ureteral obstruction (UUO)-treated rat interstitial fibrosis model

Female Sprague-Dawley rats (around 8 weeks old at the start of the experiment) were used. Under anesthesia with pentobarbital, complete ureteral obstruction of the left kidney was produced by ligating the ureter with 4-0 silk suture at two points and cutting between the ligatures. The left kidneys from each rat with UUO were harvested as obstructed kidneys and the right kidney as contralateral kidneys after perfused with phosphate buffered saline under diethyl ether anesthesia after 10 days from the operation. Harvested kidneys were fixed with 4 % (w/v) phosphate buffered paraformaldehyde for overnight and transferred to 70% ethanol. Fixed kidneys were embedded with paraffin and sectioned for immunostaining. The immunostaining of the tissue sections was carried out by using an affinity-purified rabbit anti-galectin-3 antibody as the primary antibody, a peroxidase-labeled goat anti-rabbit antibody (Sigma) as the secondary antibody, and DAB (Sigma: DAB Tablet) as the chromogenic substrate.

The result of immunostaining of the tissue sections is shown in Figure 2. The UUO model in which the overproduction and the accumulation of ECM such as collagen is observed in the interstitial region of the urinary tubule is a well known model that induces interstitial fibrosis (see Wright, E. J., et al., Lab. Invest. (1996) 74: 528-537, Yamate, J., et al., Toxicol. Pathol. (1998) 26: 793-801 and the like). In the contralateral kidneys, a small quantity of galectin-3 is present in the distal urinary tubule and the macula densa region of the glomerulus, while in the obstructed kidneys, infiltrated macrophage and fibroblasts were coexistent with galectin-3 in the fibrotic region of the tubulo-interstitium. This finding strongly suggested that galectin-3 is involved in the formation of fibrosis

because galectin-3 was coexistent with infiltrated macrophage or fibroblasts which are a kind of ECM-producing cells and are believed to induce the overproduction of ECM in the fibrotic region.

Example 3. Suppressive activity of galectin-3 on the cellular death of rat mesangium cells

Rat mesangium cells were separated from Sprague-Dawley rats according to the method of Striker et al. (Striker, G. E., et al., Lab. Invest. (1985) 53; 123-128). The separated rat mesangium cells were cultured at 37°C in the presence of 5% CO_2 in the wells of a 96-well plate using an essential medium (DMEM/F12 (1:1) culture medium containing 60 μg/ml penicillin, 100 μg/ml streptomycin, manufactured by Gibco BRL) supplemented with 10% fetal bovine serum. After culturing to semi-confluence, the cultured rat mesangium cells were washed with the essential medium, cultured for 2 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma), and then were further cultured for 1 to 4 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma) containing 0 or 50 μg/ml galectin-3 and 0 or 0.4 ng/ml TGF-β. On day 1, 2, 3, and 4 after the addition of galectin-3 and TGF-β, the amount of the cultured rat mesangium cells that survived in their respective wells were measured using as an index the conversion from MTS tetrazolium (Cell Titer 96 Aqueous one solution manufactured by Promega) to formazan by the living cells.

The result is shown in Figure 3. In both of the presence and the absence of TGF-β, galectin-3 was confirmed to suppress the cellular death of the rat mesangium cells, a kind of ECM-producing cells.

Example 4. Promoting effect of galectin-3 on the collagen type IV production by mesangium cells

Rat mesangium cells were separated in a similar

manner to that of Example 3. The separated mesangium cells were cultured at 37°C in the presence of 5% CO_2 in the wells of a 96-well plate using an essential medium (DMEM/F12 (1:1) culture medium containing 60 μg/ml penicillin, 100 μg/ml streptomycin, manufactured by Gibco BRL) supplemented with 10% fetal bovine serum. After culturing to confluence, the cultured rat mesangium cells were washed with the essential medium, cultured for 1 to 2 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma), and then were further cultured for 3 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma) containing 0, 10 or 30 μg/ml galectin-3 and 0, 0.1, 0.4 or 1.6 ng/ml TGF-β. On day 3 after the addition of galectin-3 and TGF-β, the amount of type IV collagen accumulated in the culture liquid was measured using a sandwich ELISA method that employed a goat anti-type IV collagen antibody as the immobilized antibody and a biotin-labeled goat anti-type IV collagen antibody (Chemicon) as the primary antibody. The amount of type IV collagen in the culture liquid of each well was normalized by dividing it by the amount of the living cells in each well determined in a similar manner to that described in Example 3.

The result is shown in Figure 4. It was confirmed that galectin-3 promotes the production and/or the accumulation of type IV collagen which is a kind of ECM, from the rat mesangium cells which is a kind of ECM-producing cells, in a similar manner to and in an additive manner with TGF-β.

Example 5. Inhibition of promotion by galectin-3 on collagen type IV production by rat mesangium cells, using a glycoprotein that inhibits galectin-3-binding

Rat mesangium cells were separated in a similar manner to that of Example 2. The separated rat mesangium cells were cultured at 37°C in the presence of 5% CO_2 in

wells of a 96-well plate using an essential medium (DMEM/F12 (1:1) culture medium containing 60 μg/ml penicillin, 100 μg/ml streptomycin, manufactured by Gibco BRL) supplemented with 10% fetal bovine serum. After culturing to confluence, the cultured rat mesangium cells were washed with the essential medium, cultured for 1 to 2 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma), and then were further cultured for 4 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma) containing 10 μg/ml of galectin-3 and 0, 0.1, 0.2, 0.5 or 1.5 mg/ml fetuin glycoprotein which is a substance known to inhibit galectin-3 binding (see, for example, Sato, S. et al., J. Biol. Chem. (1992) 267: 6983-6990). On day 4 after the addition of galectin-3 and fetuin, the amount of type IV collagen accumulated in the culture liquid of each well was measured using a sandwich ELISA method that employed a goat anti-type IV collagen antibody (Chemicon) as the immobilized antibody and a biotin-labeled goat anti-type IV collagen antibody (Chemicon) as the primary antibody. The amount of type IV collagen in the culture liquid of each well was normalized by dividing it by the amount of the living cells in each well determined in a similar manner to that described in Example 3.

The result is shown in Figure 5. It was confirmed that a high molecular weight glycoprotein that inhibits galectin-3 binding suppresses the promotion of the production and/or the accumulation of type IV collagen which is a kind of ECM, from the rat mesangium cells which are a kind of ECM-producing cells, by the addition of galectin-3.

Example 6. Inhibition, by galectin-3-binding inhibiting sugar, of the effect of galectin-3 on the promotion of collagen type IV production by rat mesangium cells

Rat mesangium cells were separated in a similar

manner to that described in Example 3. The separated rat mesangium cells were cultured at 37°C in the presence of 5% CO_2 in the wells of a 96-well plate using an essential medium (DMEM/F12 (1:1) culture medium containing 60 μg/ml penicillin, 100 μg/ml streptomycin, manufactured by Gibco BRL) supplemented with 10% fetal bovine serum. After culturing to confluence, the cultured rat mesangium cells were washed in the essential medium, cultured for 1 to 2 days with the essential medium supplemented with 0.1% bovine serum albumin (Sigma), and then were further cultured for 2 days in the essential medium supplemented with 0.1% bovine serum albumin (Sigma) containing 0.4 μg/ml of galectin-3 and 0, 0.25, 0.5, 1 or 2 mM of lacto-n-fucopentaose I which is, a substance known to inhibit galectin-3 binding (LNFP-1, see, for example, Sato, S. et al., J. Biol. Chem. (1992) 267: 6983-6990). On day 2 after the addition of galectin-3 and LNFP-I, the amount of type IV collagen accumulated in the culture liquid of each well was measured using a sandwich ELISA method that employed a goat anti-type IV collagen antibody (Chemicon) as an immobilized antibody and a biotin-labeled goat anti-type IV collagen antibody (Chemicon) as a primary antibody. The amount of type IV collagen in the culture medium of each well was normalized by dividing it by the amount of the living cells in each well determined in a similar manner to that described in Example 4.

The result is shown in Figure 6. It was confirmed that a low molecular weight sugar that inhibits galectin-3 binding suppresses the promotion of the production and/or the accumulation of type IV collagen which is a kind of ECM, from the rat mesangium cells which are a kind of ECM-producing cells, by the addition of galectin-3.

As hereinabove described, it was shown that galectin-3 exhibits an increased expression during pathogenesis in the anti-Thy-1.1 antibody-induced rat

nephritis model, an animal model of mesangial proliferative glomerulonephritis, (Example 1), and thus it was suggested that galectin-3 is involved in the pathogenesis of mesangial proliferative glomerulonephritis. It was also demonstrated that in the anti-Thy-1.1 antibody-induced rat nephritis model and in the obstructed kidneys of the UUO rat model, galectin-3 is coexistent with infiltrated macrophage and fibroblasts in the pre-fibrotic or fibrotic region of the tublo-interstitium (Examples 1 and 2). This finding strongly suggested that galectin-3 is involved in the formation of fibrosis because galectin-3 was coexistent with infiltrated macrophage and/or fibroblasts, a kind of ECM-producing cells, that are believed to induce the overproduction of ECM in the pre-fibrotic or fibrotic region. It was also demonstrated that galectin-3 inhibits the cellular death of the mesangium cells, a kind of ECM-producing cells (Example 3), and that it promotes the production and/or the accumulation of ECM from the ECM-producing cells (Example 4). It was further shown that a substance that inhibits the biological activity of galectin-3 suppresses the promotion of the production and/or the accumulation of ECM from ECM-producing cells by galectin-3 (Examples 5 and 6).

INDUSTRIAL APPLICABILITY

A pharmaceutical composition of the present invention comprising a compound that controls the actions of galectin-3 as active ingredient can be clinically applicable as a therapeutic or preventive agent for glomerular nephritis, diabetic nephropathy or tissue fibrosis.

CLAIMS

1. A pharmaceutical composition having an inhibitory effect on the overproduction and the accumulation of extracellular matrix, said composition comprising as an active ingredient a compound having an inhibitory effect on the biological activity of galectin-3.

2. The pharmaceutical composition according to claim 1, wherein the biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

3. The pharmaceutical composition according to claim 1 which exhibits an inhibitory effect on glomerular nephritis, diabetic nephropathy or tissue fibrosis of which cause is the overproduction and the accumulation of extracellular matrix.

4. The pharmaceutical composition according to claim 3, wherein the biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

5. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is an anti-galectin 3 antibody.

6. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is an inhibitor of galectin 3 binding.

7. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is a compound that inhibits the incorporation of galectin 3 into the cell.

8. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the transfer of galectin 3 into the nucleus.

9. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound that inhibits the biological activity of galectin-3 is a compound that inhibits the physiological activity of galectin 3 in the nucleus or the cytoplasm.

10. The pharmaceutical composition according to any of claims 1 to 4, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the expression or secretion of galectin 3.

11. The pharmaceutical composition according to any one of claims 1 to 10, which is a therapeutic or preventive agent.

12. The pharmaceutical composition according to any of claims 3 to 11, wherein the glomerular nephritis, diabetic nephropathy or tissue fibrosis is glomerular nephritis, diabetic nephropathy or tissue fibrosis, respectively, caused by the abnormal proliferation of mesangium cells.

13. The use of a compound having an inhibitory effect on the biological activity of galectin-3, for the production of a pharmaceutical composition for inhibition of the overproduction and the accumulation of extracellular matrix.

14. The use according to claim 13, wherein the biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

15. The use according to claim 13, for treatment of glomerular nephritis, diabetic nephropathy or tissue fibrosis of which cause is the overproduction and the accumulation of extracellular matrix.

16. The use according to claim 15, wherein the biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

17. The use according to any of claims 13 to 16, wherein the compound having an inhibitory effect on the

biological activity of galectin-3 is an anti-galectin 3 antibody.

18. The pharmaceutical composition according to any of claims 13 to 16, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is an inhibitor of galectin 3 binding.

19. The pharmaceutical composition according to any of claims 13 to 16, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is a compound that inhibits the incorporation of galectin 3 into the cell.

20. The pharmaceutical composition according to any of claims 13 to 16, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the transfer of galectin 3 into the nucleus.

21. The pharmaceutical composition according to any of claims 13 to 16, wherein the compound that inhibits the biological activity of galectin-3 is a compound that inhibits the physiological activity of galectin 3 in the nucleus or the cytoplasm.

22. The pharmaceutical composition according to any of claims 13 to 16, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the expression or secretion of galectin 3.

23. The use according to any one of claims 13 to 22, which is for a therapeutic or preventive use.

24. The use according to any of claims 15 to 23, wherein the glomerular nephritis, diabetic nephropathy or tissue fibrosis is glomerular nephritis, diabetic nephropathy or tissue fibrosis, respectively, caused by the abnormal proliferation of mesangium cells.

25. A method for inhibition of the overproduction and the accumulation of extracellular matrix, said method comprising administrating a compound having an inhibitory effect on the biological activity of galectin-3, to a subject which needs said inhibition.

26. The method according to claim 25, wherein the

biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

27. The method composition according to claim 25 for inhibition of glomerular nephritis, diabetic nephropathy or tissue fibrosis of which cause is the overproduction and the accumulation of extracellular matrix.

28. The method according to claim 27 wherein the biological activity of galectin-3 is to promote the production of extracellular matrix from an extracellular matrix-producing cell.

29. The method according to any of claims 25 to 28, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is an anti-galectin 3 antibody.

30. The method according to any of claims 25 to 28, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is an inhibitor of galectin 3 binding.

31. The method according to any of claims 25 to 28, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is a compound that inhibits the incorporation of galectin 3 into the cell.

32. The method according to any of claims 25 to 28, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the transfer of galectin 3 into the nucleus.

33. The method according to any of claims 25 to 28, wherein the compound that inhibits the biological activity of galectin-3 is a compound that inhibits the physiological activity of galectin 3 in the nucleus or the cytoplasm.

34. The method according to any of claims 25 to 28, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the expression or secretion of galectin 3.

35. The method according to any one of claims 25 to 34, which is for therapeutic or preventive treatment.

36. The method according to any of claims 27 to 35, wherein the glomerular nephritis, diabetic nephropathy or tissue fibrosis is glomerular nephritis, diabetic nephropathy or tissue fibrosis, respectively, caused by the abnormal proliferation of mesangium cells.

Fig.1

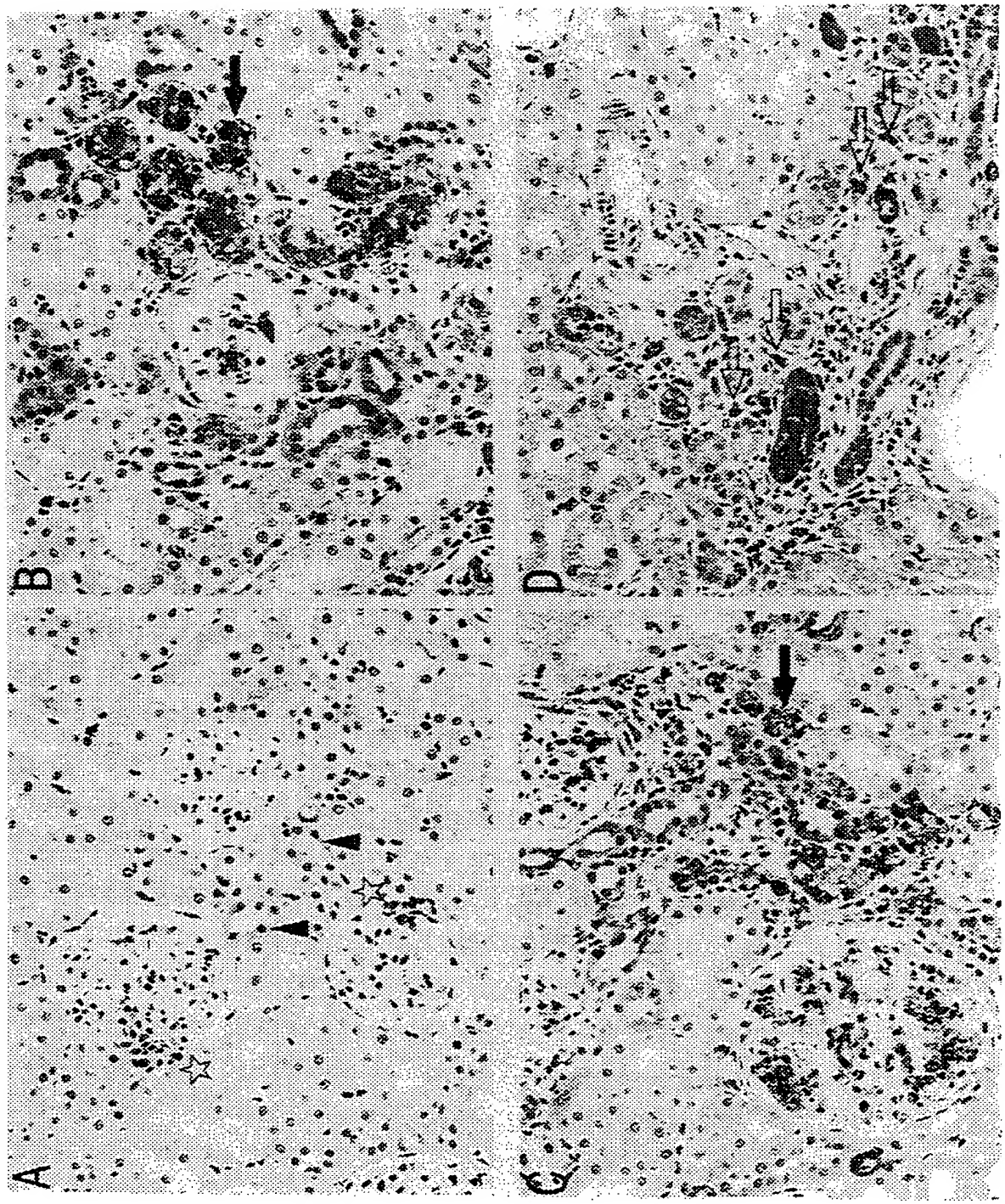


Fig.2

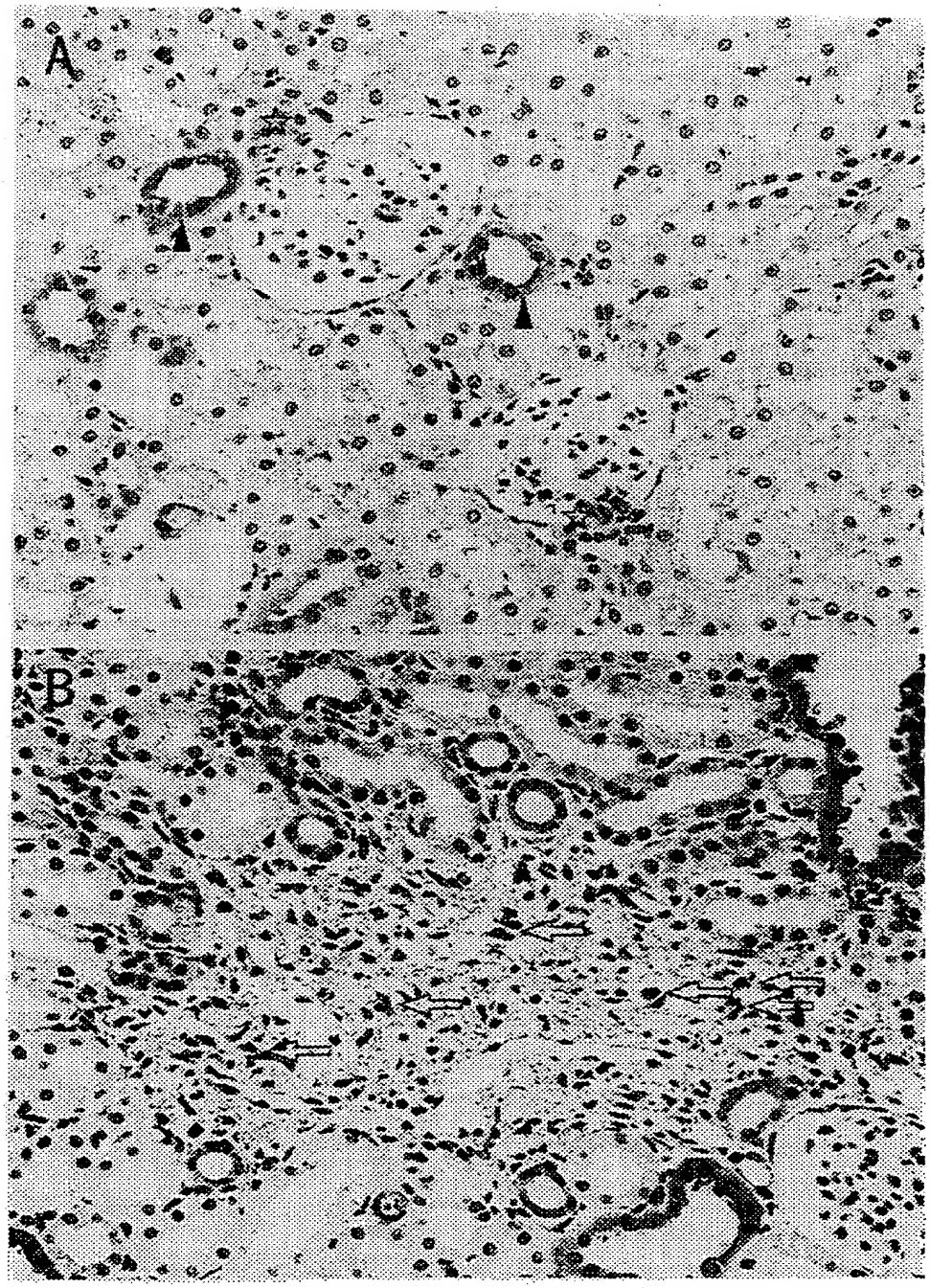


Fig.3

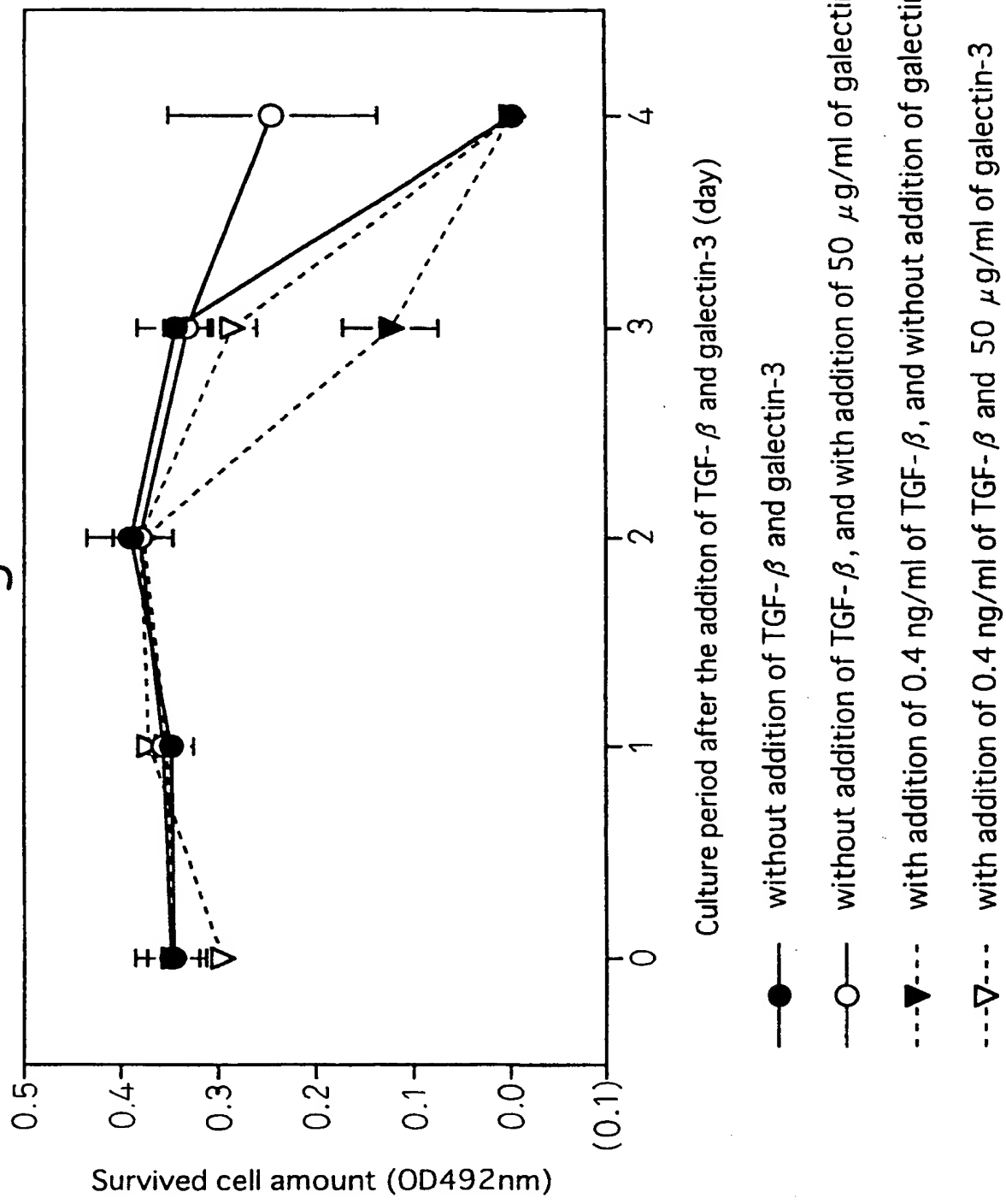
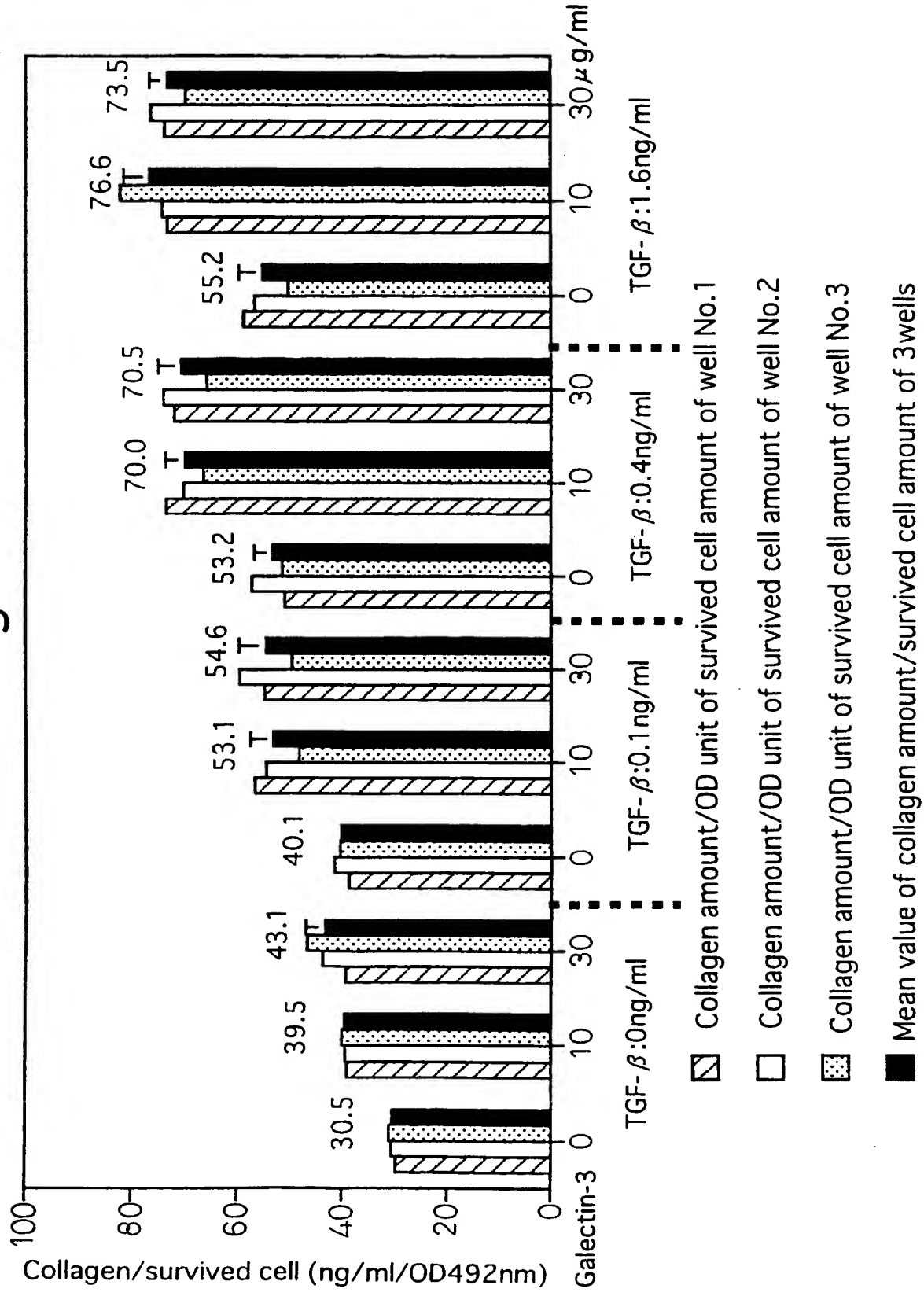

0.5
0.4
0.3
0.2
0.1
0.0
(0.1)
Survived cell amount (OD492nm)
0
1
2
3
4
Culture period after the additon of TGF-β and galectin-3 (day)
without addition of TGF-β and galectin-3
without addition of TGF-β, and with addition of 50 μg/ml of galectin-3
with addition of 0.4 ng/ml of TGF-β, and without addition of galectin-3
with addition of 0.4 ng/ml of TGF-β and 50 μg/ml of galectin-3

Fig. 4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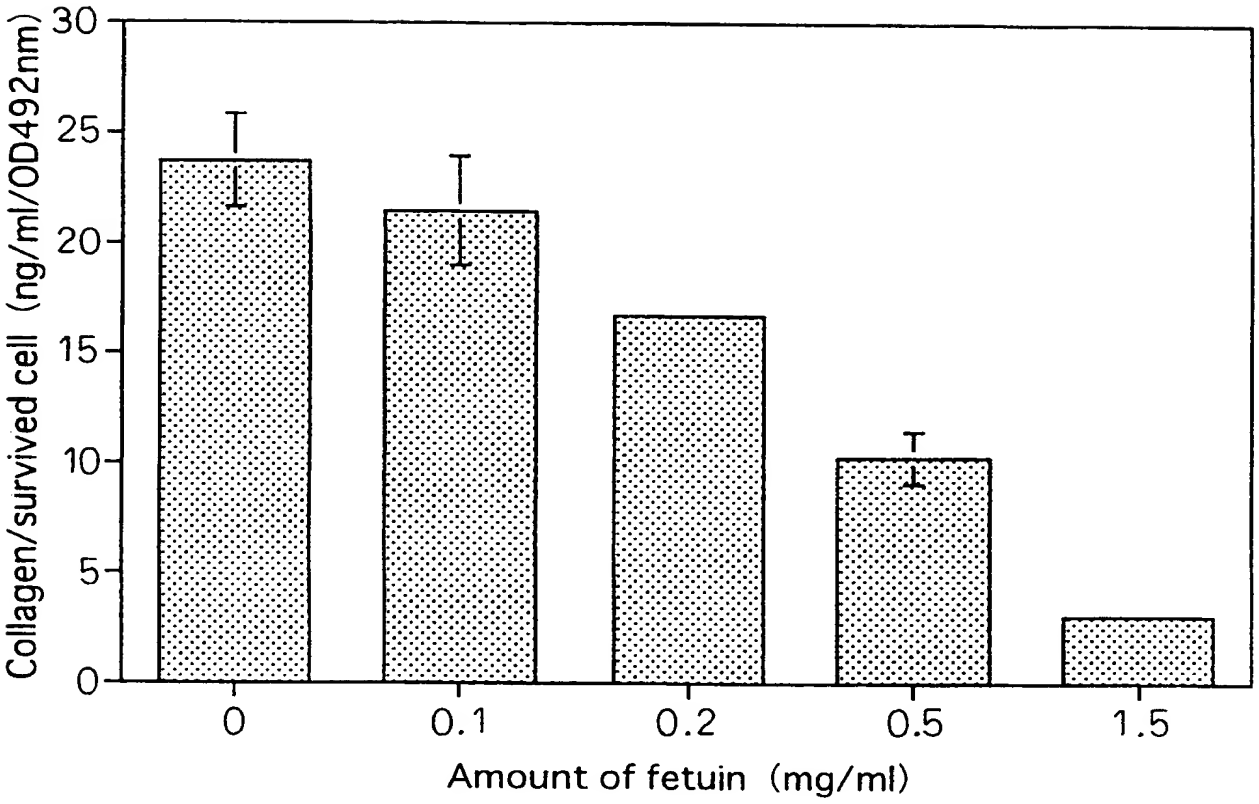
80
60
40
20
0
Galectin-3
0
10
30
30μg/ml
TGF-β:0ng/ml
TGF-β:0.1ng/ml
TGF-β:0.4ng/ml
TGF-β:1.6ng/ml
30.5
39.5
43.1
40.1
53.1
54.6
53.2
70.0
70.5
55.2
76.6
73.5
T
Collagen amount/OD unit of survived cell amount of well No.1
Collagen amount/OD unit of survived cell amount of well No.2
Collagen amount/OD unit of survived cell amount of well No.3
Mean value of collagen amount/survived cell amount of 3wells

Fig.5


Collagen/survived cell (ng/ml/OD492nm)
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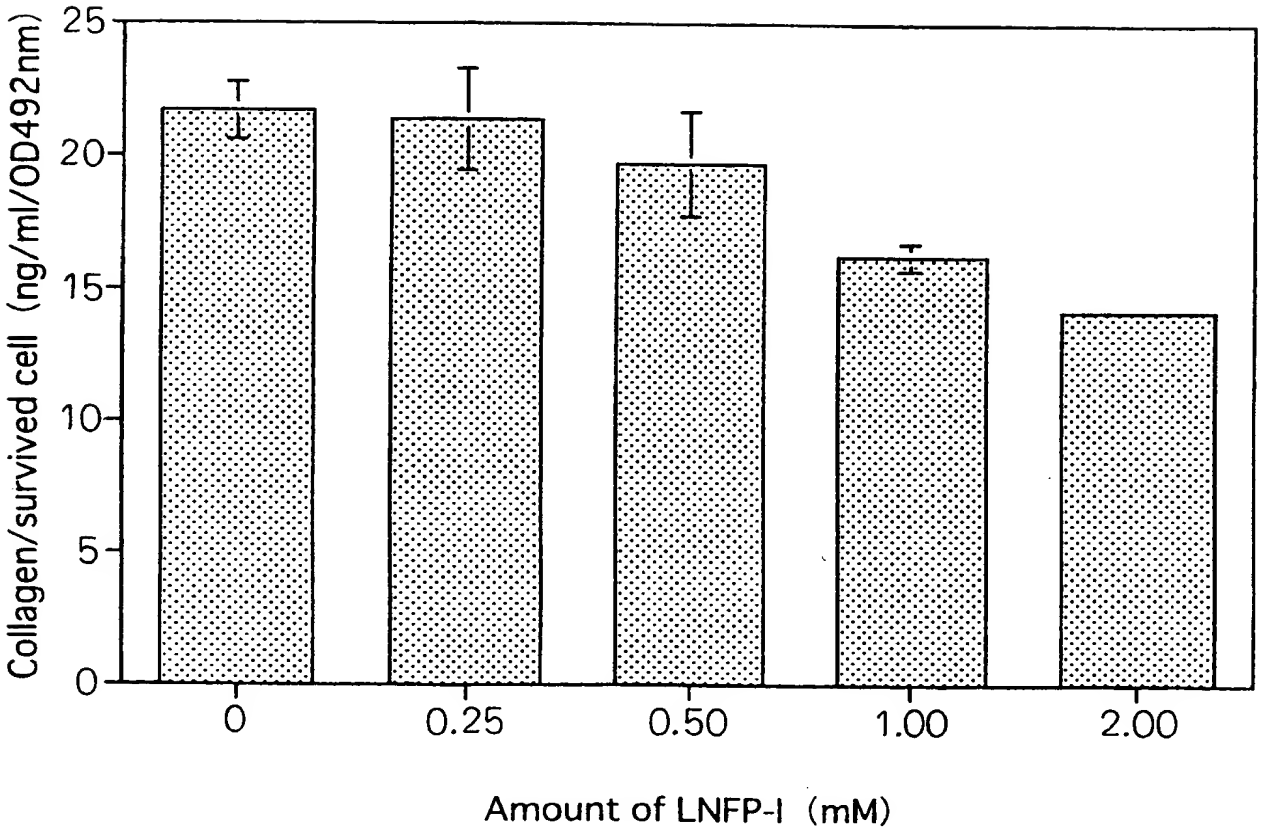
25
20
15
10
5
0
0
0.1
0.2
0.5
1.5
Amount of fetuin (mg/ml)

Fig. 6


Collagen/survived cell (ng/ml/OD492nm)
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20
15
10
5
0
0
0.25
0.50
1.00
2.00
Amount of LNFP-I (mM)

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(57) Abstract

A pharmaceutical composition having an inhibitory effect on the overproduction and the accumulation of extracellular matrix, said composition comprising as an active ingredient a compound that inhibits the biological activity of galectin–3, which pharmaceutical composition can serve as a therapeutic or preventive agent for glomerular nephritis, diabetic nephropathy or tissu fibrosis, as well as the use of said compound for the production of pharmaceuticals for the above–mentioned use, and a method for inhibition of the overproduction and accumulation of the extracellular matrix.

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CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
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Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OCHIENG J ET AL: "Regulation of cellular adhesion to extracellular matrix proteins by galectin-3." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 MAY 29) 246 (3) 788-91. , XP002131316 the whole document	1-36
A	HENRICK K ET AL: "Evidence for subsites in the galectins involved in sugar binding at the nonreducing end of the central galactose of oligosaccharide ligands: sequence analysis, homology modeling and mutagenesis studies of hamster galectin-3." GLYCOBIOLOGY, (1998 JAN) 8 (1) 45-57. , XP000876954 the whole document	1-36

-/--

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 February 2000	16/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/JP 99/04238

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROBSTMEIER R ET AL: "Galectin-3, a beta-galactoside-binding animal lectin, binds to neural recognition molecules." JOURNAL OF NEUROCHEMISTRY, (1995 JUN) 64 (6) 2465-72. , XP000876914 the whole document	1-36
A	LIU F T ET AL: "Modulation of functional properties of galectin -3 by monoclonal antibodies binding to the non-lectin domains." BIOCHEMISTRY, (1996 MAY 14) 35 (19) 6073-9. , XP002131317 cited in the application the whole document	1-36

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 99/04238

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 25-36 are directed to a method of treaetment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

PATENT COOPERATION TREATY

210

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

ISHIDA, Takashi
A. AOKI, ISHIDA, & ASSOCIATES
Toranomon 37 Mori Bldg., 5-1,
Toranomon 3-chome, Minato-Ku
TOKYO 105-8423
JAPON

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	31.08.2000
Applicant's or agent's file reference G888-PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP99/04238	05/08/1999	06/08/1998

Applicant
TEIJIN LIMITED et. al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Danti, B Tel.+49 89 2399-8161

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
G888-PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP99/04238	05/08/1999	06/08/1998
International Patent Classification (IPC) or national classification and IPC A61K39/395		
Applicant TEIJIN LIMITED et. al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/03/2000	31.08.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Knudsen, H Telephone No. +49 89 2399 8696

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/JP99/04238

I. Basis of th report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-16	as originally filed	

Claims, No.:

1-16,17 (part),26 (part), 27-36		as originally filed
17 (part),18-25, 26 (part)	with telefax of	11/08/2000

Drawings, sheets:

1/6-6/6	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.

☒ claims Nos. 25-36.

because:

☒ the said international application, or the said claims Nos. 25-36 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	17-21,29-33
	No:	Claims	1-16,22-27,34,36
Inventive step (IS)	Yes:	Claims	17-18,29-30
	No:	Claims	1-16,19-21,22-27,31-34,36
Industrial applicability (IA)	Yes:	Claims	1-24
	No:	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see s parate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

ITEM III:

Claims 25-36 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

ITEM V:

NOVELTY & INVENTIVE STEP:

5.1 Claim 1 is defined as being a composition which comprises a compound having an inhibitory effect on the biological activity of galectin-3. According to dependent claim 6, the compound may inhibit binding of galectin-3. Dependent claim 7 shows that the compound may inhibit incorporation of galectin-3 into a cell. Claim 8 mentions that the compound may inhibit the transfer to the nucleus in the cell. Claim 9 states that the compound could inhibit the activity of galectin-3 and claim 10 describes the compound as being an inhibitor of the expression or secretion of galectin-3.

Thus, it is clear that the inhibitor as defined in claim 1 covers an enormous number of compounds with a high variety of physiological activities which are not necessarily specific for galectin-3, but may regulate transport protein in the cells or permeability of cell membranes (cf the present application's description p.6, line 11 - page 8, line 15). As mentioned in the cited passage, compounds with these characteristics are already known from the prior art, eg anti-galectin-3 antibodies which inhibit the binding of galectin-3 to cells are known from "Biochemistry, vol 35(19), p.6073-6079, (1996)" (D1). Novelty therefore cannot be acknowledged for claims 1 and 5-10.

5.2 The definition of the biological activity of galectin-3 (claims 2 and 4), of the disease in which galectin-3 is involved (claims 3 and 12) and of the time of administering the agent (claim 11) does not add any technical features to the subject-matter of claim 1 and are therefore considered to lack novelty as well.

5.3 Considering the broad definition of "compound having an inhibitory effect on the biological activity of galectin-3" which is practically unlimited as shown above, it is expected that the definition encompasses the compounds used conventionally for the treatment of overproduction of ECM. The applicant argues that this argumentation is not based on existing facts. However, the IPEA is of the opinion that galectin-3 is overproduced at injury sites (see application's description p.3, l.29-30) and that the immune suppressive agents used for the conventional treatment of these injuries will therefore inhibit the production of galectin-3. Thus, at least claims 13-16, 22-23, 25-27 and 34 therefore do not appear to be novel over the prior art cited on page 3, lines 22-28.

5.4 The examples of inhibitors of :

(1) the incorporation of galectin-3 into the cell;
(2) the transfer of galectin-3 into the nucleus; and
(3) the physiological activity of galectin-3 in the cytoplasm or nucleus

mentioned in the description pages 7-8 have not been suggested in the prior art for treatment of diseases characterized by overproduction of ECM. Thus, claims 19-21 and 31-33 are considered novel, at present. However, it is expected, though not demonstrable at present, that compounds used in the conventional treatment of ECM-related diseases have these effects especially as the effects are broadly defined in the claims.

5.5 Nevertheless, given the complexity of the intracellular physiological processes and the lack of test data, it is not clear whether the said inhibitors solve any technical problem and claims 19-21 and 31-33 therefore are not considered inventive.

5.6 The diseases listed in claims 24 and 36 appear to be caused by abnormal proliferation of mesangium cells in most cases (cf the description, page 3, lines 3-10). Thus, claims 24 and 36 lack novelty for the same reasons as claims 15 and 27.

5.7 However, the use of anti-galectin-3 antibody or saccharides for inhibition of the overproduction of extracellular matrix (ECM) is not disclosed in the cited prior art documents and claims 17-18 and 29-30 therefore appear to be novel.

5.8 In the prior art documents, it is disclosed that the binding of galectin-3 to cells has a number of effects in cell growth regulation (see D1, p.6073, right column and "Journal of Neurochemistry, vol.64(6), pages 2465-2472, (1995)" (D2) page 2470, right column). Among the effects described are formation of aggregates and activation of mast cells, neutrophils, and monocytes. For the skilled person, who knows from the prior art that galectin-3 has these effects, it would appear to be obvious that an inhibitor of the binding of galectin-3 to cells would cause a certain on the activity of the cell. However, there is nothing in the prior art which shows that the use of binding agents for galectin-3 can be used for reducing the collagen production from mesenchymal cells and therefore may be used for the treatment of diseases characterised by an overproduction of ECM. Claims 17-18 and 29-30 therefore appear to be inventive.

INDUSTRIAL APPLICABILITY:

5.9 For the assessment of the present claims 25-36 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

5.10 The subject-matter of claims 1-24 is considered industrially applicable.

ITEM VII:

Contrary to the requirements of Rule 5(a)(ii) PCT, D2 is not identified in the description and the relevant background art disclosed therein is not briefly discussed.

ITEM VIII:

8.1 As indicated in above Item V, the definition of the active compound comprised in the claimed pharmaceutical composition of claim 1 and used in the method claims 13 and 25 is very broad. The physiological effects used for defining the compound are so vaguely defined that the skilled person cannot determine precisely which

compounds fall within the said definition. The applicant argues that it is easy to determine whether a given compound falls within the scope given in claim 1. The IPEA does not agree, because "biological activity" encompasses a high number of different physiological activities which must all be tested and no specific tests are given in the application.

8.2 The definitions in the dependent claims, apart from claims 5, 11, 17, 23, 29 and 36 are all based on physiological effects. The said definitions suffer from lack of clarity for the same reasons as the definition used in the independent claims.

8.3 The present invention is based on the discovery that a glycoprotein (fetuin) and a sugar (lacto-n-fucapentaose) are capable of inhibiting the galectin-3 induced overproduction of collagen from in-vitro cultured mesangium cells. However, the description does not contain any evidence that a treatment with a galectin-3 inhibitor has a preventive or therapeutic effect on ECM overproduction. Considering the high complexity of the cell regulating processes, it does not appear to be possible to predict the effect of a galectin-3 inhibitor on the basis of the in-vitro data presented in the application. Thus the claims are not fully supported by the description.

biological activity of galectin-3 is an anti-galectin 3 antibody.

18. (Amended) The use according to any of claims 13 to 16, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is an inhibitor of galectin 3 binding.

19. (Amended) The use according to any of claims 13 to 16, wherein the compound having an inhibitory effect on the biological activity of galectin-3 is a compound that inhibits the incorporation of galectin 3 into the cell.

20. (Amended) The use according to any of claims 13 to 16, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the transfer of galectin 3 into the nucleus.

21. (Amended) The use according to any of claims 13 to 16, wherein the compound that inhibits the biological activity of galectin-3 is a compound that inhibits the physiological activity of galectin 3 in the nucleus or the cytoplasm.

22. (Amended) The use according to any of claims 13 to 16, wherein the compound that inhibits the biological activity of galectin-3 is a compound that modulates the expression or secretion of galectin 3.

23. The use according to any one of claims 13 to 22, which is for a therapeutic or preventive use.

24. The use according to any of claims 15 to 23, wherein the glomerular nephritis, diabetic nephropathy or tissue fibrosis is glomerular nephritis, diabetic nephropathy or tissue fibrosis, respectively, caused by the abnormal proliferation of mesangium cells.

25. A method for inhibition of the overproduction and the accumulation of extracellular matrix, said method comprising administrating a compound having an inhibitory effect on the biological activity of galectin-3, to a subject which needs said inhibition.

26. The method according to claim 25, wherein the

14 U.S. 09/744328

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP99/04338	11/08/1999	12/08/1998

International Patent Classification (IPC) or national classification and IPC
A61K31/67

Applicant
TAKEDA CHEMICAL INDUSTRIES, LTD. et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
VI	☒	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/09/1999	20.12.2000
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Simm, M.D. Telephone No. +49 89 2399 7411

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/JP99/04338

I. Basis of th report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-46 as originally filed

Claims, No.:

1-20 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-13, 19, 20 .

because:

☒ the said international application, or the said claims Nos. 19 in respect of i.a. relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-13, 19, 20 (all partial).

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-14, 16, 19, 20
Inventive step (IS)	Yes:	Claims	see Sep. sheet
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-14, 16, 20

No: Claims 19

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re It m I

Basis of the opinion

Reference is made to the following documents:

D1: WO 96 39134 A (HOSHINO TETSUO ;IWASA SUSUMU (JP); MURANISHI HIROYA (JP); TAKEDA C) 12 December 1996 (1996-12-12)
D2: WO 98 08517 A (HOSHINO TETSUO ;IWASA SUSUMU (JP); SAITO KAZUHIRO (JP); TAKEDA CHE) 5 March 1998 (1998-03-05)
D3: EP-A-0 719 782 (TAKEDA CHEMICAL INDUSTRIES LTD) 3 July 1996 (1996-07-03)
D4: EP-A-0 460 488 (TAKEDA CHEMICAL INDUSTRIES LTD) 11 December 1991 (1991-12-11)
D5: ODA, TSUNEO ET AL: 'Synthesis of Novel 2-Benzothiopyran and 3-Benzothiepin Derivatives and Their Stimulatory Effect on Bone Formation' J. MED. CHEM. (1999), 42(4), 751-760, July 1999 (1999-07), XP000867476
D6: AKIYAMA H ET AL: 'TAK-778, a novel synthetic 3-benzothiepin derivative, promotes chondrogenesis in vitro and in vivo.' BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1999 JUL 22) 261 (1) 131-8.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

No International Preliminary Examination will be carried out in respect of subject-matter which is not covered by the search report (Rule 66.1(e) PCT).

Claim 19 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The opinion expressed as to novelty, inventive step and industrial applicability only refers to matter for which an international search report has been drawn up (see also Item III), i.e. the use of certain benzothiepine or benzothiopirane in treating bone diseases.

Novelty (Art. 32(2) PCT).

Compounds of formula I and II are disclosed in D1 and D2. Compounds falling within the scope of formula III are disclosed in D1-D2 (see page 8, line 26 in D1 and page 10, line 36 in D2) and in D4 Compound 41 (on page 37) is the compound of claim 8 of the present application. D3 discloses the compounds of formula II and the compounds of claim 6 of the present application. The compound subject-matter of claim 7 is disclosed in D1 (see for instance claim 15 of D1).
Furthermore, D1-D4 disclose the use of these compounds in pharmaceutical compositions for the treatment or prevention of bone diseases (see Abstracts).

Thus, the subject-matter of claims 1-14, 16, 19 and 20 is not novel.

It appears that the only new information provided in this application is the discovery that the enhancement of cell differentiation have a direct influence on BMP on in other words the further characterization of known benzothiepine or benzothiopirane as "enhancers of cell differentiation induction factors".
In this respect, the following should be noted: the use o a compound for the manufacture of a medicament for the treatment of a specified disease, once known, can not be patented under the guise of another or newly specified pharmacological mechanism.
In fact, the discovery of such a new way of action or mechanism is not an invention as the technical effect obtained remains the same, and a new therapeutic window is not opened. Moreover, the further characterization of known compounds does not render the compounds novel.

Industrial Applicability (Art. 33(4) PCT).

For the assessment of the present claim 19 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VI

Certain documents cited

Documents D5 (2.6.99) and D6 (22.07.99) might become relevant if the priority is not valid.

Re Item VIII

Certain observation on the international application

The expression "an enhancer of cell differentiation induction factor" in the claims is not clear and does not characterised the compounds to which it refers (Art. 6 PCT).

The "**benzothiopirane**" claimed appear to lack support by the description (Examples) (Art. 6 PCT).

PATENT COOPERATION TREA[illegible]

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 25 April 2000 (25.04.00)	
International application No. PCT/JP99/04238	**Applicant's or agent's file reference** G888-PCT
International filing date (day/month/year) 05 August 1999 (05.08.99)	**Priority date** (day/month/year) 06 August 1998 (06.08.98)
Applicant SASAKI, Satoshi et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

02 March 2000 (02.03.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Forax
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38